IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Group Art Unit 1639

In re

Patent Application of

Allan M. Tereba, et al.

Application No. 10/694,475

Confirmation No.: 4550

Filed: October 27, 2003

Examiner: Christopher M. Gross

"SIMULTANEOUS ISOLATION AND QUANTITATION OF DNA"

Electronically filed by:

~~Sally Sorensen~~ Tracy Bruesewitz

12/28/09

Date

REPLY BRIEF UNDER 37 C.F.R. §41.37

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This Reply Brief is submitted in response to the Examiner's Answer mailed on October 27, 2009. Because December 27, 2009 fell on a Sunday, this Reply Brief should be considered as having been timely filed on Monday, December 28, 2009. The Applicant respectfully requests consideration of the following remarks in response to the Examiner's Answer. Please charge or credit Deposit Account No. 50-0842 with any fee that may be associated with this communication.

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REAL PARTY IN INTEREST

The real party in interest is Promega Corporation, the assignee of record.

RELATED APPEALS AND INTERFERENCES

None.

STATUS OF CLAIMS

Claims 1-43 are canceled. Claims 44-82 are currently pending; claims 55-57, 59 and 71 are withdrawn from consideration; claims 44-54, 58, 60-70, and 72-82 were rejected in an Office Action mailed November 18, 2008. The rejection of claims 44-54, 58, 60-70, and 72-82 is appealed.

STATUS OF AMENDMENTS

The amendment submitted with the response of February 17, 2009 was entered.

SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 44, and its dependent claims 45-65 and 82, are directed to methods for isolating a defined and consistent amount of DNA from multiple samples (p. 9, lines 21-25; p. 30, line 1-p. 31, line 4) comprising selecting a defined amount of DNA to be isolated from the samples (p. 10, lines 26-30); choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample (p. 6, line 34-p. 7, line 1; p. 17, lines 10-13); contacting each sample with the discrete amount of the silica-containing solid support (p. 6, lines 16-19), each sample comprising DNA in excess of the binding capacity of the discrete amount of silica-containing solid support (p. 7, lines 7-8), under conditions that allow reversible binding of the defined amount of DNA to the solid support (p. 6, lines 23-24); and separating each sample from the support to isolate a defined and consistent amount of DNA from each sample (p. 6, lines 21-23).

Independent claim 66, and its dependent claims 67, 68, and 77-81, are directed to methods of isolating DNA from multiple samples for use in a molecular biological procedure (p. 9, lines 10-20, and lines 31-33) comprising selecting a defined amount of DNA to be isolated from the samples (p. 5, lines 20-21); choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample (p. 6, lines 34-p. 7, line 1; p. 17, lines 10-13); contacting each sample with a discrete amount of a silica-containing solid support (p. 6, lines 16-19), each sample comprising DNA in excess of the binding capacity of the discrete amount of silica-containing solid support (p. 7, lines 7-8), under conditions that allow reversible binding of the defined amount of DNA to the solid support (p. 6, lines 23-24); and eluting bound DNA to isolate a defined and consistent amount of DNA from each sample (p. 6, lines 21-23), wherein the eluted DNA is suitable for use in the molecular biological procedure (p. 9, lines 10-20).

Independent claim 69, and its dependent claims 70-76, are directed to kits for isolating a defined and consistent amount of a DNA from multiple samples according to the claimed methods, the kits comprising silica magnetic particles, a discrete amount of which is used with each sample, the discrete amount having the capacity to reversibly bind a defined amount of the DNA from each sample, the samples comprising DNA in excess of the binding capacity of the discrete amount of silica magnetic particles (p. 7, line 29-p. 8, line 2).

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issues presented for consideration by the Board are as follows:

1. Whether the Examiner has established a legally sufficient case for rejecting claims 44, 45, 50, 53, 66, 67, and 82 under 35 U.S.C. § 102(b) as being anticipated by Melzak et al. (1996 J. Colloid and Interface Sci. 181:635-644), as evidenced by information available at http://seq.yeastgenome.org/.

2. Whether the Examiner has established a legally sufficient case for rejecting claims 44-54, 58, 60-65, 67, 68, and 77-82 under 35 U.S.C. § 103(a) as being unpatentable over Melzak et al., in view of Kleiber et al. (WO 96/41811).

3. Whether the Examiner has established a legally sufficient case for rejecting claims 69, 70, and 72-76 under 35 U.S.C. § 103(a) as being unpatentable over Melzak et al., in view of Kleiber et al., and in further view of Ryder et al. (U.S. Patent No. 5,639,599).

REPLY TO EXAMINER'S ANSWER

Grounds of Rejection to be Reviewed upon Appeal

The Examiner's Answer indicates that Appellants' statement of the grounds of rejection to be reviewed upon Appeal is "substantially correct", except that the Examiner asserts that claims 44-54, 58, 60-70, 72-82 are rejected under 35 USC 103(a) as being unpatentable over Melzak in view of Kleiber and further in view of Ryder. (See section (6) of Examiner's Answer, pp 1-2). However, this characterization of the grounds of rejection is not supported by either the record (see Office action mailed November 18, 2008, p. 9, first paragraph; and Advisory Action, mailed May 1, 2009, p. 3, first paragraph), or by the Examiner's Answer itself (see Examiner's Answer, pp 6-8).

The Examiner's Answer further confuses the grounds of rejection in its response to Appellants' arguments regarding anticipation. Although the Examiner's Answer apparently agrees with Appellants' statement of grounds for rejection with respect to the alleged anticipation of claims by Melzak (Section (6) of Examiner's Answer, pp 1-2), the Examiner's Answer states that "appellant argues Melzak does not anticipate the claims. The rejection, however, is for obviousness, not anticipation." (See *id.*, p. 10, penultimate paragraph).

The problems attendant even the most seemingly simple and straightforward of tasks, i.e., identifying the rejections of record, underscores the confusion and disjointedness that has characterized the protracted examination of this application.

Response of Examiner's Answer to Appellants' Argument

Anticipation

In response to Appellants' argument that Melzak adds nothing beyond the teachings of Smith, which the Examiner has acknowledged does not anticipate or render obvious the claimed invention, the Examiner's Answer stated that Smith is not relevant, because the current rejections are over Melzak. The point Appellants attempted to make is that Melzak is cumulative to Smith. After the Examiner acknowledged that Smith does not anticipate the claimed invention and withdrew that rejection, he found another reference, Melzak, which teaches nothing more than Smith. Melzak, like Smith, describes a DNA binding curve for silica material. Whereas Smith used a plot of DNA bound to or eluted from a solid support as a function of total DNA to show

results of experiments comparing the relative binding capacities of different types of silica-containing materials, Melzak used similar plots in the context of experiments conducted to study the "dominant driving forces affect involved in DNA adsorption to silica in perchlorate solutions". In contrast to the Examiner's characterization, Melzak, like Smith, fails to teach the steps of selecting a defined amount of DNA to be isolated from the samples and then choosing a discrete amount of a silica containing solid support necessary to isolate the defined amount of DNA from each sample.

Examiner's Answer characterizes section 10 of the [Bitner] declaration as an *admission* by Appellant that Greenspoon & Ban perform an experiment analogous to that shown in Fig. 3 of Melzak. Fig. 3 of Melzak shows that linear DNA remains bound to the particles (Fig. 3a) and that essentially no supercoiled plasmid DNA is bound to the particles (Fig. 3b) upon dilution with 6M perchlorate. In point of fact, the Greenspoon & Ban publication referred to in section 10 of the Bitner Declaration does not present the results of a saturation curve. Rather, Greenspoon & Ban, which is not prior art to the instant application, describes isolating consistent amounts of DNA from mock sexual assault samples made from either ½, ¼, or 1/8 of a swab containing semen diluted 1:10, 1:100, or 1:200 using the DNA IQ System, a kit used in accordance with the method of the invention (Bitner Declaration, paragraph 5). As a consequence of being able to isolate consistent amounts of DNA from samples having an 80-fold variation in the amount of DNA, there were no noticeable differences in DNA amplified from the samples using PowerPlex1.1 (See Fig. 2 of Greenspoon & Ban).

Appellants' argument that Melzak does not teach all elements of the claimed invention in as much as it fails to teach selecting a defined amount of DNA to be isolated from multiple samples and then choosing a discrete amount of silica-containing solid support needed to isolate the defined amount of DNA from the samples is dismissed by the Examiner. In discounting Appellants' position, the Examiner is impermissibly reading elements out of the claims.

Obviousness

Appellants argued that the combination of references do not combine to teach all of the claim limitations, in as much as the secondary references do not cure the deficiencies of the primary reference.

In addition to attacking the prima facie case of obviousness, Appellants provided evidence of secondary considerations that support the non-obviousness of the invention by way

of a declaration under 37 CFR 1.132 by Dr. Rex Bitner ("the Declaration") and attachments to the Declaration, identified in the Declaration as Exhibits B-E, which were submitted in a response filed October 31, 2007.

The evidence of secondary considerations presented includes evidence of contemporaneous appreciation of those skilled in the art. As noted in the Declaration, DNA IQ™ System refers to a product of Promega Corporation that employs a method for isolating a defined and consistent amount of DNA from multiple samples according to the claimed invention. Evidence of contemporaneous appreciation for the claimed invention by those skilled in the art includes Promega's having been honored with an R&D 100 Award in 2002 for its DNA IQ™ System (Declaration, paragraph 16).

The Examiner's Answer conflates the praise received by the claimed invention, as embodied by the DNA IQ™ System, with commercial success, and argues that according to MPEP 716.03(b), an applicant must show that the claimed features were responsible for the commercial success of an article. The basis for the Examiner's conclusion that contemporaneous appreciation and commercial success are equivalent is Appellants' citation to case law recognizing that appreciation by contemporaries in the field of invention is a useful indicator of whether the invention would have been obvious at the time it was made. See *Vulcan Eng'g Co. v. Fata Aluminum, Inc.* 278 F.3d 1366, 1373 (Fed. Cir. 2002). The Examiner erroneously concluded that the quotation from the Federal Circuit decision in Vulcan concerned only commercial success. In fact, Vulcan also held that the District Court did not err in considering contemporaneous recognition of achievements of the Vulcan system, including articles in trade journals and testimony of witnesses concerning the belief in the engineering community that the lost foam process could not be effectively mechanized as a continuous on-line process. Appreciation by contemporaries skilled in the field of the invention is a useful indicator of whether the invention would have been obvious to such persons at the time it was made. *Id.*

The purpose of MPEP 716.03(b) is to provide guidance for distinguishing commercial success owing to the claimed invention from commercial success owing to extraneous factors, such as advertising or promotion. However, being honored with an R&D 100 Award is not evidence of commercial success, but rather, is evidence of contemporaneous appreciation by those skilled in the art.

The US Department of Energy Office of Science website numbers among its accomplishments providing support for 800 projects that received an R&D 100 Award from 1962-2008. The website describes the R&D 100 Awards as follows:

> Widely recognized in industry, government, and academia as a mark of excellence for the most innovative ideas of the year, the R&D 100 Awards are the only industry-wide competition rewarding the practical applications of science.
>
> The R&D 100 Awards recognize the most promising new products, processes, materials, or software developed throughout the world and introduced to the market the previous year. Awards are based on each achievement's technical significance, uniqueness, and usefulness compared to competing projects and technologies.
>
> (See Attachment 2).
>
> The US Department of Energy Office of Electricity Delivery & Energy Reliability declares that "winning of an R&D 100 Award provides a mark of excellence known to industry, government, and academia as proof that the product is one of the 100 most technologically significant new products of the year."
>
> (See Attachment 3).

The Examiner questions the selection criteria for the R&D 100 Awards, and whether Promega's receipt of an R&D 100 Award for the DNA IQ™ System should be accorded substantial weight. As noted in the Declaration, the DNA IQ™ System is a commercial embodiment for carrying out the claimed invention (Declaration, paragraph 5).

Additionally, the claimed methods were issued as a patent in Europe and were recently allowed in Japan (See Attachments 4 and 5). While the United States Patent and Trademark Office is not bound by decisions of other jurisdictions regarding the patentability of claims, the European and Japanese Patent Offices' allowance of the claims certainly validate the decision of R&D Magazine in its conclusion regarding the uniqueness of the claimed invention.

The Examiner's Answer states that evidence presented in the Bitner declaration does not satisfy criteria for establishing addressing a long felt need in the industry, as set forth in MPEP 716.04. The Examiner acknowledges that Appellants have provided evidence sufficient to establish the need for improving DNA sample throughput. However, with regard to the second prong, failure of others, the Examiner asserts that Appellant admits in the Declaration that prior approaches did not isolate a defined and consistent amount of DNA from multiple samples, but rather, focused on maximizing yield (Declaration, paragraph 6). With all due respect, if

establishing that an invention addresses a long felt need requires providing evidence that others had tried and failed *to provide the same solution to the problem*, one would never be able to establish long felt need without running afoul of some section of 35 USC 102 or 35 USC 112. In other words, interpreting evidence of the "failure of others" to mean evidence that others have tried and failed to use *Appellants' solution* to meet the long felt need is completely illogical.

With regard to the third prong of actually satisfying the long felt need, the Examiner argues that it is unclear whether improved DNA sample throughput is due to the DNA IQ™ System or to the Beckman Coulter Biomek® 2000 Laboratory Automation Workstation. While a robotic system like Beckman Coulter Biomek® 2000 Laboratory Automation Workstation contributes to the increased throughput, it is generally concerned with automation of liquid-handling procedures (Attachment 6), whereas the DNA IQ™ System is required to allow recovery of consistent amounts of DNA. The Greenspoon and Ban article (Exhibit B of the Declaration) demonstrates that the DNA IQ™ System can be implemented using a robotic system such as the Biomek® 2000 Laboratory Automation Workstation to yield consistent amounts of DNA from vastly disparate samples. As noted in the letter from Mr. Jeffrey Ban (Exhibit E of the Declaration), the adaptability of the DNA IQ™ System allows automation of purification of consistent amounts of DNA. The Biomek® 2000 Workstation existed as part of the prior art before the availability of the DNA IQ™ System, and did not by itself satisfy the long felt need. Thus, the method embodied by the DNA IQ™ System was the essential advance of the art needed for satisfying the long felt need.

Appellants note that the Examiner completely ignores any evidence establishing that the DNA IQ™ System satisfies a long felt need. The Examiner makes no mention of Exhibits C and D of the Declaration. Exhibit C is a letter from Dr. Weimin Sun, who was Scientific Director of the Molecular Genetics Department at Quest Diagnostics Nichols Institute. In his letter, Dr. Sun describes the DNA IQ™ System as being highly tolerant to the variability of sample input quantity and as demonstrating high consistency in the quantity of product DNA, which are highly desirable in light of significant variations in starting material contained in buccal swabs.

Exhibit D of the Declaration is a letter from Kim Gorman, President of Paternity Testing Corporation. Ms. Gorman describes historic problems with working with buccal swabs in a 96-well automated format, and that DNA IQ™ allows for extractions to be carried out in a 96-well format. As Ms. Gorman explains, DNA IQ™ has the advantage of eliminating the need to

quantify DNA, because the concentration of DNA obtained is consistent from well to well. Ms. Gorman further notes that the DNA IQ™ System is much faster than most extraction systems that were tried using the 96-well format (30 min. vs. 6-7 hours).

The correspondence attached to the Declaration not only establishes that DNA IQ™ System addresses a long felt need, but also provides further evidence of contemporaneous appreciation by those of skill in the art.

Conclusions

For the foregoing reasons, claims 44-54, 58, 60-70, and 72-82 should be allowed. Further, as generic claim 44 is allowable, withdrawn claims 55-57 and 59, which depend directly or indirectly from claim 44, should be rejoined and allowed. Similarly, as claim 69 is allowable, withdrawn claim 71, which depends from claim 69, should be rejoined and allowed. Appellants respectfully request that the Board reverse the rejections and pass the application to allowance.

Respectfully submitted,

Date: December 28, 2009

Jill A. Fahrlander
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CLAIMS APPENDIX

1.-43. (Cancelled)

44. (Previously presented) A method for isolating a defined and consistent amount of DNA from multiple samples comprising:

(a) selecting a defined amount of DNA to be isolated from the samples;

(b) choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample;

(c) contacting each sample with the discrete amount of the silica-containing solid support, each sample comprising DNA in excess of the binding capacity of the discrete amount of silica-containing solid support, under conditions that allow reversible binding of the defined amount of DNA to the solid support; and

(d) separating each sample from the support to isolate a defined and consistent amount of DNA from each sample.

45. (Previously presented) The method of claim 44, further comprising:

(e) separating the DNA of step (d) from the support.

46. (Previously presented) The method of claim 44, wherein the silica-containing solid support comprises silica magnetic particles.

47. (Previously presented) The method of claim 46, wherein the silica magnetic particles are porous.

48. (Previously presented) The method of claim 46, wherein the silica magnetic particles are nonporous.

49. (Previously presented) The method of claim 46, wherein the silica magnetic particles are siliceous-oxide coated magnetic particles.

50. (Previously presented) The method of claim 44, wherein the conditions comprise the presence of a chaotropic salt.

51 (Previously presented) The method of claim 50, wherein the chaotropic salt comprises guanidine thiocyanate.

52. (Previously presented) The method of claim 44, wherein the DNA is genomic DNA.

53. (Previously presented) The method of claim 44, wherein the DNA is plasmid DNA.

54. (Previously presented) The method of claim 44, further comprising analyzing the defined amount of DNA of step (d).

55. (Withdrawn) The method of claim 44 wherein the sample comprises a solid support.

56. (Withdrawn) The method of claim 55 wherein the solid support of the sample is paper.

57. (Withdrawn) The method of claim 55, wherein the solid support of the sample is a swab.

58. (Previously presented) The method of claim 44 wherein the sample is a forensic sample.

59. (Withdrawn) The method of claim 55, wherein the sample is contacted with a chaotropic salt.

60. (Previously presented) The method of claim 59, wherein the contacted sample is heated to a temperature of from about 60° to about 100°C.

61. (Previously presented) The method of claim 44, further comprising determining at least a portion of the sequence of the isolated DNA.

62. (Previously presented) The method of claim 45, further comprising washing the solid support prior to step (e).

63. (Previously presented) The method of claim 62, wherein the solid support is washed with a solution comprising an alcohol and a salt.

64. (Previously presented) The method of claim 45, wherein the DNA of step (e) is separated by eluting with water.

65. (Previously presented) The method of claim 50, wherein the concentration of chaotropic salt is between about 0.1 M and 7 M.

66. (Previously presented) A method of isolating DNA from multiple samples for use in a molecular biological procedure comprising:

(a) selecting a defined amount of DNA to be isolated from the samples;

(b) choosing a discrete amount of a silica-containing solid support necessary to isolate the defined amount of DNA from each sample;

(c) contacting each sample with a discrete amount of a silica-containing solid support, each sample comprising DNA in excess of the binding capacity of the discrete amount of silica-containing solid support, under conditions that allow reversible binding of the defined amount of DNA to the solid support; and

(d) eluting bound DNA of step (c) to isolate a defined and consistent amount of DNA from each sample, wherein the eluted DNA is suitable for use in the molecular biological procedure.

67. (Previously presented) The method of claim 66, wherein the molecular biological procedure includes analysis of at least one DNA sequence comprising at least one short tandem repeat sequence.

68. (Previously presented) The method of claim 67, wherein the at least one short tandem repeat sequence comprises the Combined DNA Index System loci.

69. (Previously presented) A kit for isolating a defined and consistent amount of a DNA from multiple samples according to claim 44, the kit comprising:

silica magnetic particles, a discrete amount of which is used with each sample, the discrete amount having the capacity to reversibly bind a defined amount of the DNA from each sample, the samples comprising DNA in excess of the binding capacity of the discrete amount of silica magnetic particles.

70. (Previously presented) The kit of claim 69 wherein the sample comprises blood.

71. (Withdrawn) The kit of claim 69, wherein the sample comprises a solid support.

72. (Previously presented) The kit of claim 69, further comprising a chaotropic salt.

73. (Previously presented) The kit of claim 69, wherein the silica magnetic particles are provided in a solution comprising the chaotropic salt.

74. (Previously presented) The kit of claim 69 further comprising a wash solution.

75. (Previously presented) The kit of claim 69, wherein the silica magnetic particles are siliceous oxide-coated magnetic particles.

76. (Previously presented) A kit for isolating a defined and consistent amount of DNA from multiple samples according to claim 66, the kit comprising, silica magnetic particles, a discrete amount of which is used with each sample, the discrete amount having the capacity to reversibly bind a defined amount of the DNA from each sample, the samples comprising DNA in excess of the binding capacity of the discrete amount of silica magnetic particles.

77. (Previously presented) The method of claim 66, wherein the procedure is a DNA amplification reaction.

78. (Previously presented) The method of claim 66, wherein the procedure is a DNA sequencing reaction.

79. (Previously presented) The method of claim 66, wherein the procedure is a DNA nucleic acid hybridization.

80. (Previously presented) The method of claim 66, wherein the DNA of step (d) is eluted in a discrete volume to provide a solution having a defined DNA concentration suitable for use in the procedure without separate quantification.

81. (Previously presented) The method of claim 80, wherein the DNA concentration is from about 0.5 ng/μl to about 5.0 ng/μl and the procedure is a DNA amplification reaction.

82. (Previously presented) The method of claim 44, wherein the defined and consistent amount of DNA isolated is within 60% to 229% of the mean amount of DNA isolated from the samples.

EVIDENCE APPENDIX

1. Attachment 1: Declaration of Rex Bitner under 37 C.F.R. 1.132, including Exhibits A-E attached thereto – (submitted with response of October 31, 2007 and with Appeal Brief of July 17, 2009).

2. Attachment 2: U.S. Department of Energy Office of Science website printout.

3. Attachment 3: U.S. Department of Energy Office of Electricity Delivery & Energy Reliability website printout.

4. Attachment 4: European Patent 1204741 consisting of cover page and English version of granted claims.

5. Attachment 5: Correspondence from Japanese associate with official Decision of Grant and translation of allowed claims.

6. Attachment 6: Description of Beckman Coulter Biomek® 2000.

RELATED PROCEEDINGS APPENDIX

None.

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant(s):	Tereba et al.	**Docket No.:**	016026-9043-US01
Serial No.:	10/694,475	**Group Art Unit:**	1639
Filing Date:	October 27, 2003	**Examiner:**	Christopher M. Gross
Title:	SIMULTANEOUS ISOLATION AND QUANTITATION OF DNA		

DECLARATION OF REX BITNER UNDER 37 CFR § 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Rex Bitner, do hereby declare and state the following:

1. I have served as the Technology Manager of the Genetic Analysis R&D group at Promega Corporation, Madison, WI, since 2003. From 1997-2003, I worked as a Senior Scientist and Senior Project Manager of R&D at Promega Corporation. I hold a B.S. in Biology from The University of Washington, Seattle, WA and a Ph.D. in Genetics from The University of California, Davis, CA. A copy of my curriculum vitae is attached as Exhibit A.

2. I am a joint inventor of at least some of the claimed subject matter of the above-identified patent application. I make this declaration in support of prosecution of the present application before the U.S. Patent and Trademark Office.

3. I have read and understand the invention as disclosed in the present application, including the invention described by the presently pending claims. I have also reviewed the July 31, 2007 Office Action. I understand that each of claims 44-52, 54, 55, and 58-68 is rejected as being unpatentable (i.e., obvious) over Kleiber et al. (WO 96/41811), Huber et al. (1993 Nuc. Acids Res. 21:1061-1066), and Vogelstein et al. (1979 PNAS 76:615-619).

4. Claims 44-52, 54, 55, and 58-68 are directed to methods of isolating a defined and consistent amount of DNA from multiple samples by choosing the amount of DNA to be

ATTACHMENT 1

isolated, choosing an amount of a silica containing solid support needed to isolate the defined amount of DNA, such that the amount of DNA in the samples is greater than the binding capacity of the solid support, and contacting each sample with the solid support under conditions that allow isolation of the defined and consistent amount of DNA.

5. DNA IQ™ System is the term that Promega Corporation, the assignee of the instant application, uses with its customers when referring to methods for isolating a defined and consistent amount of DNA from multiple samples, as described throughout the application and as summarized in paragraph 4, above.

6. The concept underlying the methods of the invention, which focus on isolating just a portion of DNA that may be present in a sample, represents a complete departure from prior art methods such as those described in Kleiber et al. and Vogelstein et al., which focus on maximizing DNA yield. For example, Kleiber et al. discusses the relatively high yields obtained by their methods. (Please see, for example, page 12 of Kleiber et al.). Similarly, Vogelstein et al. emphasizes that binding DNA to glass from dissolved agarose "is rapid, convenient, and nearly quantitative." (Vogelstein et al., p. 618, first column, last line to second column, first line, emphasis added). Huber et al., rather than isolating a defined and consistent amount of DNA, describes using high performance liquid chromatography (HPLC) to fractionate relatively small (i.e., <500 bp) DNA fragments such as restriction fragments and PCR products according to size.

7. The ability to isolate a defined and consistent amount of DNA from multiple samples using the methods of the invention simplifies processing, reduces the amount of time needed to process samples, and increases sample throughput. Because DNA samples prepared using the method of the invention contain a select defined and consistent amount of DNA, the isolated DNA can be used directly in downstream applications requiring an amount of DNA within a particular range.

8. The importance of increased sample throughput in isolating DNA cannot be overstated. For example, because prior art methods of isolating DNA from samples were very labor-intensive and cost about $1000 per test, a backlog of several hundred thousand samples from rape victims awaited processing in 1999 when the patent application for the present invention

ATTACHMENT 1

was submitted. Thus, samples containing evidence that may have been useful in identifying sex offenders go unprocessed.

9. Attached as Exhibit B is an article entitled "Robotic Extraction of Mock Sexual Assault Samples using Biomek® 2000 and the DNA-IQ™ System", authored by Susan Greenspoon and Jeff Ban of the Virginia Division of Forensic Science, which appeared in the February 2000 issue of Profiles in DNA.

10. Greenspoon and Ban describe how the DNA IQ™ System, used in conjunction with the Biomek® 2000 robotics system, was able to isolate uniform amounts of DNA from mock sexual assault samples containing dilutions of semen of from 1:10 to 1:200 on a ½, ¼, or 1/8 portion of a swab (See p. 4, Mock Sexual Assault Samples). The DNA thus isolated from samples having widely varying DNA content was used directly in PowerPlex® 1.1 System, a DNA amplification reaction used in genetic identity testing, and produced uniform results.

11. Other experts in the field have recognized the importance of the instantly claimed methods isolating DNA for use in molecular biological methods such as amplification for genetic identity testing.

12. Dr. Weimin Sun, Scientific Director in the Molecular Genetics Department of Quest Diagnostics Nichols Institute, San Juan Capistrano, CA, evaluated the DNA IQ™ System for use in clinical samples and found that the methods yielded consistent quantities of DNA despite significant variations in the starting material, which afforded satisfactory performance in downstream applications. (See correspondence form Dr. Sun to Mr. David Phelps, Exhibit C).

13. Kim Gorman, then President of Paternity Testing Corporation, Columbia, MO, reported that the DNA IQ™ System was used to extract DNA from buccal swabs in a 96 well format. (See correspondence from Ms. Gorman to Mr. Phelps, Exhibit D). Ms. Gorman noted that buccal swabs vary greatly in DNA content, and also noted that DNA multiplexes (amplification of multiple DNA loci in a single reaction) are concentration sensitive. Despite these challenges, Ms. Gorman further noted that there was no need to quantify the DNA prepared using the DNA IQ extraction method prior to use in a multiplex reaction. Ms. Gorman reported that, using the DNA IQ™ System, the total time required to process 96 samples was reduced from 6 or 7 hours

ATTACHMENT 1

using their traditional extraction method to about 3 hours, and hands on time by analysts was reduced from more than 4 hours to less than 30 minutes.

14. Jeffrey Ban, Section Chief of Forensic Biology at the Virginia Department of Criminal Justice Services, Division of Forensic Science, reported that, using the DNA IQ™ System in conjunction with Beckman Coulter Biomek® 2000 Workstation or other similar instrumentation, could greatly increase a laboratory's throughput capabilities, thus permitting the Forensic DNA community to provide better service to law enforcement agencies (See correspondence from Mr. Ban to Mr. Phelps, Exhibit E).

15. In addition, Promega has been contacted by numerous law enforcement agencies that ultimately used the DNA IQ™ System to analyze forensic samples from crime scenes. For example, the Royal Canadian Mounted Police requested assistance in processing samples collected from a hog farm in British Columbia, where the partial remains of at least 26 murder victims were found. The DNA IQ™ System was also used to isolate DNA for genetic identity testing of victims at Ground Zero and human remains found in mass graves in Bosnia.

16. In 2002, Promega received an R&D 100 Award for its DNA IQ™ System. Through the R&D 100 Awards program, sponsored by R&D Magazine, organizations receiving the awards are recognized for the most technologically significant products introduced into the marketplace.

17. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: October 30, 2007

Rex Bitner

Rex Bitner

Q:\CLIENT\016026\9043\B1171888.1

ATTACHMENT 1

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QUALIFICATIONS SUMMARY

Over twenty years of industrial experience in biotechnology research and product development at Promega (Madison, WI), Amersham/Pharmacia Biotech (now GE Healthcare), (Milwaukee, WI), and 3M (St. Paul, MN). Four years of postdoctoral research in molecular biology at the University of Colorado, Boulder and the University of California, Davis. Broad, in-depth knowledge of molecular biology, solid phase purification of biological materials, and all aspects of product development in an ISO9001: 2000 environment. Experienced in technology management, project leadership and management, supervision of a BL3 laboratory, and the development, launch and care for biotechnology products. Extensive experience in the creation, development and evaluation of intellectual property, including USPTO and foreign patent office actions, in person interviews with USPTO patent examiners, as well as participation in foreign patent opposition proceedings, both as proprietor and as an opposition party, and as an opposition member in the appeal of an invalidated patent.

PROFESSIONAL HISTORY

1997 - present **PROMEGA CORPORATION, Madison, Wisconsin**

Technology Manager, Genetic Analysis, R&D (2003 - present)

Technology Manager, Genetic Analysis: management of a laboratory group, development of separation technologies, intellectual property, and purification products for the biotechnology marketplace and clinical laboratory market, with particular emphasis on automation of nucleic acid purification products for use in genomics, high throughput pharmaceutical drug screening, and clinical diagnostics. Intellectual property management has included USPTO and foreign patent office actions, in person interviews with USPTO patent examiners, phone interviews with USPTO and foreign patent examiners, and participation in European patent opposition proceedings, both as proprietor and as an opposition party, including as an opposition member in the appeal of an invalidated patent. The efficient integration of foreign patent filing strategies and licensing of intellectual property within Promega's Genetic Analysis business strategy has been a central responsibility.

Senior Scientist, Senior Project Manager, R&D (1997 - 2003)

Development of pH dependent ion exchange purification systems using both column and paramagnetic particle purification methods in robotic workstations, with additional emphasis on automated cell concentration and magnetic clearing of cellular lysates in 96-well walkaway automated DNA purification (particularly using Beckman BioMek® FX and Tecan Genesis® robotic platforms). Products developed for genomic DNA purification from human whole blood and tissues (including R&D Magazine's R&D 100 award winning DNA-IQ™(for 2002), plant materials, and DNA purification from food ingredients for use in the quantitative detection of genetically modified organisms (GMO) in food. Additional experience with DNA sequencing automation, RNA purification, and PCR cleanup, particularly using Beckman, Tecan and Thermo-Electron Labsystems robotic platforms.

Project leader for Promega products, in an ISO9001: 2000 environment, including:

Wizard® Genomic, 10ml blood	A1620	Wizard® Magnetic DNA Purification for Food	FF3751
Wizard® Magnetic 96 DNA Plant	FF3761	MagneSil® Blood Genomic, Max Yield	MD1360
MagneSil® ONE, Fixed Yield	MD1370	MagneSil® KF, Genomic System	MD1460
PureYield™ RNA Midi-Prep System	Z3741	PureYield™ Plasmid Midi-Prep System	A2495

PROFESSIONAL HISTORY, continued

1995 - 1997 **AMERSHAM PHARMACIA BIOTECH INC., Milwaukee, Wisconsin.**

Senior Research Scientist (1995 - 1997)

Senior Research Scientist and Project Leader responsible for nucleic acid purification products for the biotechnology laboratory: development of novel separations matrices and processes, formulation and execution of project plans, maintenance of timelines and scheduling, and supervision of personnel on several project teams. Research and development of new products for the molecular biology marketplace, particularly in the areas of proprietary purification products, anion exchange chromatography, and solid phase extraction and immobilization of nucleic acids. Responsibilities included the management of personnel, timelines and ISO 9001 documentation of product development.

1982 - 1994 **3M COMPANY, St. Paul, Minnesota.**

Research Specialist (1984 - 1994)

Identifying, planning and pursuing molecular biology programs of interest to 3M businesses. Research programs involved diverse product objectives: Development of 3M's Rapid Attest™ biological/sterilization monitor product, GMP purification of bovine phosphophoryn proteins (for bone repair), genetic manipulation of bacteria to produce specialty chemicals (*meta*-hydroxyphenylacetylene, and aromatic compounds useful in laser dyes), cDNA cloning of mammalian genes for drug discovery screening, surface immobilization of nucleic acids onto ceramic oxide/3M Empore™ (PTFE) membranes (for use in DNA blotting, hybridization and sequencing), solid phase extraction of DNA for automated sequencing, solid phase extraction of DNA from human blood plasma for use in PCR, protein immobilization on azlactone functionalized porous beads (including 3M Emphaze™ beads and network beads), and cloning of stress protein genes from bacteria associated with periodontal disease. Development and implementation of DNA purification technologies, using a variety of ceramic matrices for solid phase extractions. Over ten years experience supervising a biosafety level 3 containment laboratory.

Other responsibilities included evaluation of both internal and external research proposals and intellectual property. Additional responsibilities as Institutional Biosafety Officer, OSHA blood-borne pathogen safety officer, and as scientific advisor in the development of and compliance with Minnesota State (Environmental Quality Board) regulations governing recombinant organisms.

Senior Biologist (1982 -1984)

Responsibilities included setting up and staffing a recombinant DNA laboratory, evaluation of outside business proposals and intellectual property issues, and initiation of new research programs in molecular biology: gene expression in *Bacillus subtilis*, and R&D of 3M's Rapid Attest™. Additional responsibilities: initiation of university research contracts and management of laboratory personnel.

1978 - 1982 **UNIVERSITY OF COLORADO, Boulder, Colorado.**

Postdoctoral research: Dr. Peter L. Kuempel, Dept. of Molecular, Cellular, and Developmental Biology: Termination of chromosome replication in *E. coli*.

PROFESSIONAL HISTORY, continued

1974 - 1978 **UNIVERSITY OF CALIFORNIA, Davis, California.**

Postdoctoral research: Dr. Gordon G. Edlin, Dept. of Genetics (1978).
Instructor: Department of Genetics (1977).

1974 **UNIVERSITY OF WASHINGTON, Seattle, Washington**
Post-graduate Research Assistant: Dr. Jonathan A. Gallant, Dept. of Genetics

EDUCATION

Ph.D. in Genetics, 1978
University of California, Davis, California

B.S. in Biology, *cum laude*, 1974
University of Washington, Seattle, Washington

PATENTS

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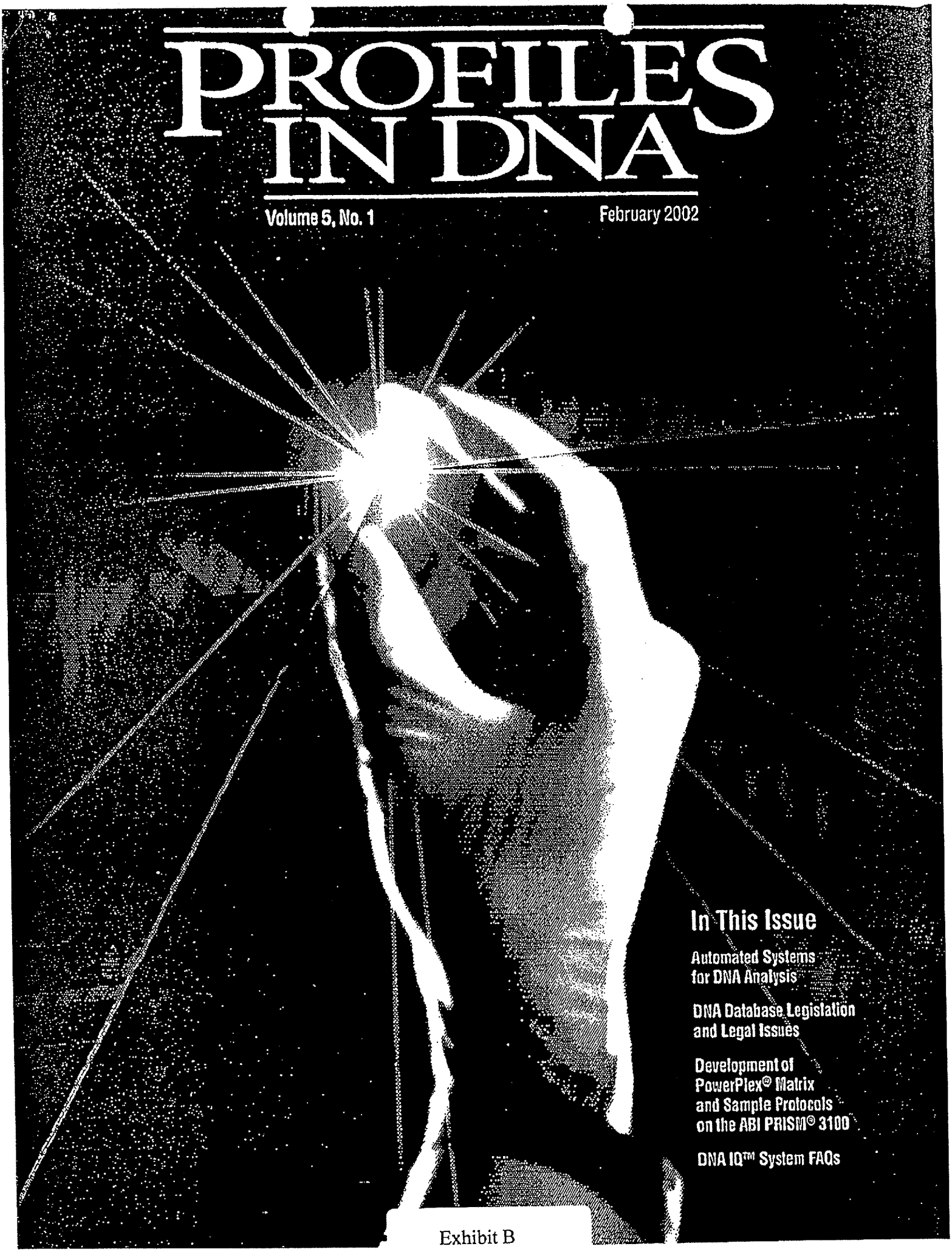
PROFILES IN DNA

Volume 5, No. 1 February 2002



In This Issue

Automated Systems for DNA Analysis

DNA Database Legislation and Legal Issues

Development of PowerPlex® Matrix and Sample Protocols on the ABI PRISM® 3100

DNA IQ™ System FAQs

Exhibit B

Robotic Extraction of Mock Sexual Assault Samples Using the Biomek® 2000 and the DNA IQ™ System

By Susan Greenspoon and Jeff Ban
Virginia Division of Forensic Science, Richmond, Virginia

The Biomek® 2000 used in conjunction with the DNA IQ™ System has proven to be an efficient robotic system designed to handle the challenges of routine casework samples.

INTRODUCTION

Forensic scientists are routinely faced with the challenge of isolating DNA from a large array of tissue and cell types. The variety of substrates upon which cellular material has been deposited, some of which may contain inhibitors of PCR, can make the process more difficult (1). Therefore, any robotic system applied to the extraction of forensic casework samples must be robust enough to address these variations. The Biomek® 2000 used in conjunction with the DNA IQ™ System(a) has proven to be an efficient robotic system designed to handle the challenges of routine casework samples.

The DNA IQ™ System uses silica-coated magnetic beads to separate DNA from cellular debris. Cells are lysed in a powerful lysis buffer, and the lysate is mixed with the magnetic beads. The beads saturate at approximately 100ng of bound DNA, and the excess DNA is removed by pipetting. Once bound to the magnetic resin, the DNA is pipetted and vigorously shaken several times in wash buffer, then eluted using heat. The Biomek® 2000 is equipped with a magnetic plate, a shaking platform and a thermal exchange unit to perform these necessary steps.

CONTAMINATION STUDIES

A number of exploratory and validation studies have been performed on the Biomek® 2000/ DNA IQ™ System to evaluate the viability of this automated system for use with forensic samples. Fundamental questions needed to be answered before moving ahead with extensive validation work. First, does the robotic, open-plate format cause contamination? To answer this question, two sample formats were used repeatedly, and extracted samples were analyzed to test

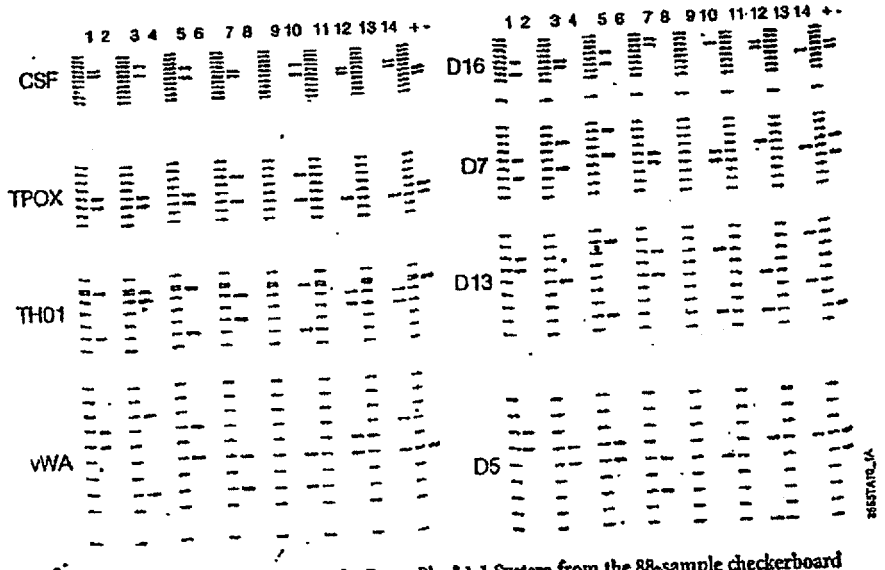


Figure 1. Amplified DNA samples using the PowerPlex® 1.1 System from the 88-sample checkerboard contamination study. Numbers 1–14 indicate sample numbers, "+" indicates positive control (K652), and "–" indicates negative control. Amplified DNA was analyzed by electrophoresis using a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585nm scan (left panel) and the 505nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO® Instrument).

for contamination. The first is the zebra-stripe format test: alternating columns of samples containing an abundant source of DNA with columns containing reagent blanks (8 sample wells per column). Therefore, a column of samples containing abundant DNA was processed adjacent to a column of reagent blanks in a striped pattern on the plate. The samples containing DNA were bloodstains cut into 5mm² squares. DNA was eluted from the magnetic beads into 100µl of sterile water, then quantified, amplified, and typed using the PowerPlex® 1.1 System[b,c] (2). The first two trials of this test detected some contamination. The software method used was modified to accommodate sample loading into a 96 deep-well plate in place of the more shallow Greiner plate and to remove an initial shaking step. A subsequent zebra stripe experiment showed no contamination with the 40 samples that were isolated.

The second contamination test is a checkerboard sample format: samples containing abundant DNA were alternated with reagent blanks in a checkerboard pattern across a 96 deep-well plate (Figure 1). All 128 samples (88 sample and 40 sample methods) tested negative for any detectable contamination.

MOCK SEXUAL ASSAULT SAMPLES

Sexual assault cases frequently constitute the majority of DNA cases received by a forensic laboratory. Presently, no robotic system is available that can separate sperm from non-sperm cells and thus perform a differential extraction (3) from start to finish. However, the first step of separating fractions can be performed manually. Subsequently, the DNA from E-cell (epithelial or non-sperm cell) lysates and sperm pellets can be extracted robotically, saving analysts a substantial amount of time. Any robotic extraction of sexual assault samples must at least be able to generate sample DNA, equivalent in both quality and yield to that generated by manual DNA extraction methods. Therefore, a thorough examination of the Biomek® 2000/DNA IQ™ System's ability to isolate DNA from sexual assault samples needed to be performed. The first step was to ascertain whether sperm cells could be successfully lysed and the DNA purified by the robotic system. Mock sexual assault samples were prepared using previously donated vaginal swabs and semen from a known donor, which was deposited onto sterile cotton swabs in 1:2 and 1:4 dilutions. The E-cells were lysed manually, the sperm cells pelleted, and a portion of the lysates and the entire sperm pellets were loaded onto the Biomek® 2000 for DNA extraction. DNA was eluted off the magnetic beads into 100µl of sterile water. High-quality DNA was obtained and typed accurately using the PowerPlex® 1.1 System (data not shown).

Once it was demonstrated that the Biomek® 2000/DNA IQ™ System could successfully complete the differential extraction process with the E-cell lysates and intact sperm, the next question addressed was whether this automated system could produce DNA of comparable quality and yield to that produced by manual extraction. A comparative study was designed to measure the performance of the Biomek® 2000/DNA IQ™ System with respect to manual extraction of similar if not identical samples. Samples were prepared in the following manner:

1. Sets of vaginal swabs from 5 different donors were selected.
2. Duplicate mock sexual assault swabs were prepared using semen from a single donor at the following dilutions: 1:10, 1:100, 1:1,000 and 1:10,000 for three sets, 1:10, 1:100, 1:200 and 1:400 for one set and 1:100, 1:200, 1:400 and 1:800 for the last set.
3. Once dried, the swabs were cut into 1/2, 1/4 and 1/8 portions.
4. The E-cells were lysed and the sperm cells pelleted and washed.
5. The samples were split evenly with one half going to an analyst to complete the extraction manually and the other loaded onto the Biomek® 2000 for a robotic DNA extraction.

Yields and quality of the DNA from the sperm fractions processed by the Biomek® 2000/DNA IQ™ System were comparable and frequently superior to those obtained by manual extraction (Figure 2). In less experienced hands, the Biomek® 2000/DNA IQ™ System clearly outperformed the manual extraction (data not shown). Results obtained by experienced users were equivalent to those achieved with the robot. Therefore, the Biomek® 2000/DNA IQ™ System is not only capable of outperforming its human counterpart, but it also delivers a more consistent product.

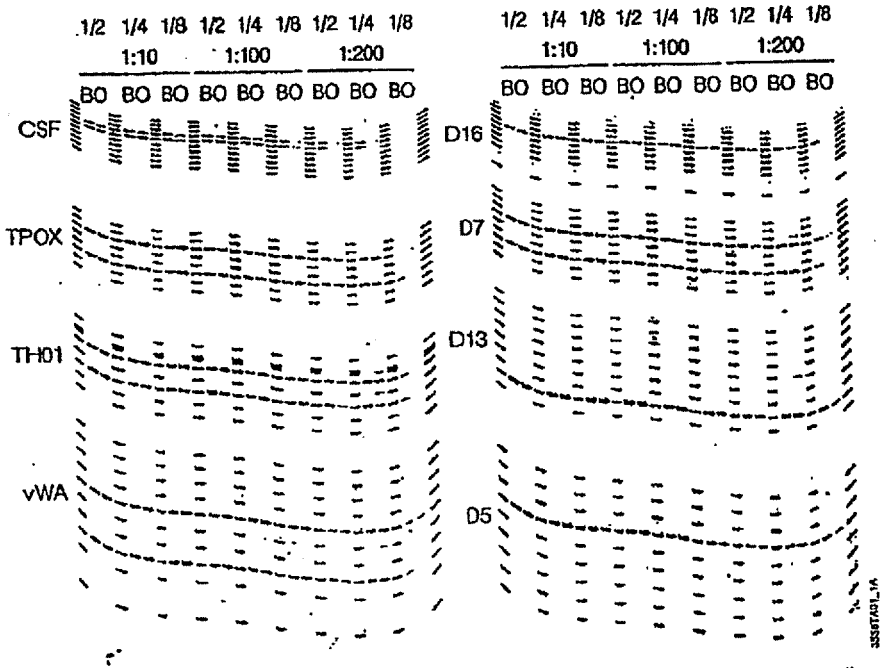


Figure 2. PowerPlex® 1.1 mock sexual assault comparison study. Duplicate samples were extracted manually (indicated by an "O" above the lane, for organic) or robotically (indicated by a "B" above the lane, for Biomek® 2000). Semen dilutions (1:10, 1:100 and 1:200) for each set of swabs are indicated above the corresponding six sample lanes, which contain DNA extracted from the indicated swab portion (1/2, 1/4 or 1/8). Amplified DNA was separated by electrophoresis in a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585nm scan (left panel) and the 505nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO® instrument).

The maximum sample volume for use with the 96 deep-well plate is limited to 100µl. Sperm cells are typically in a pellet of 50µl and therefore unaffected by the volume limit. Since the E-cell lysate is usually in a volume of 500µl, it was important that the yields from 100µl of E-cell lysate from the 1/2, 1/4 and 1/8 swab portions be sufficient for all DNA typing needs. Total yields of E-cell DNA extracted on the robot were calculated (Figure 3), and sufficient E-cell DNA could be obtained using robotic extraction methods. In fact, 1/8 swab portions provided more than enough DNA for an E-cell DNA profile.

The amount of time saved by using the Biomek® 2000/DNA IQ™ System to extract the forensic samples can be substantial. The time it takes to complete the organic extraction manually for a single sample is 5 hours and 5 minutes (after E-cells have been lysed, and sperm cells pelleted and washed). Of course, additional samples will lengthen the amount of time proportionately. In comparison, the robot takes 1 hour and 15 minutes to extract the DNA from 40 samples and 1 hour and 50 minutes to extract 88 samples. Therefore, the absolute minimum amount of time saved is 3 hours and 50 minutes or half of a day.

EXTRACTION OF OTHER CELL AND TISSUE TYPES

Since a variety of cells and tissue types are encountered in routine forensic casework, the Biomek® 2000/DNA IQ™ System was evaluated to determine its capability to isolate DNA from a variety of sources. Dried bloodstains, E-cell lysates, intact sperm cells, muscle, heart, brain, liver and buccal swabs were extracted using the Biomek® 2000/DNA IQ™ System and successfully typed using the PowerPlex® 1.1 System (data not shown).

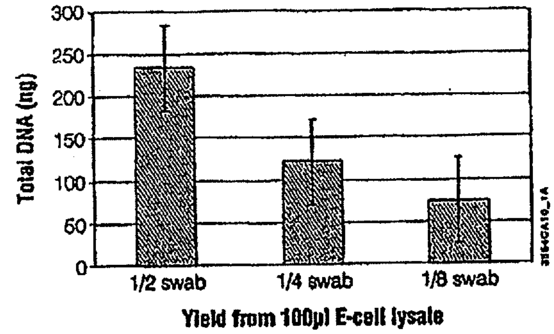


Figure 3. Bar graph depicting the total yield of E-cell DNA generated from extraction on the Biomek® 2000 robot using 100µl of lysate. Yields were determined by measuring DNA concentration using the QuantiBlot® kit.

CONCLUSION

A completely automated system for extraction of sexual assault samples is currently not available. However, once the E-cells have been separated from the sperm cells, robotic DNA extraction using the Biomek® 2000/DNA IQ™ System can be accomplished. Moreover, when sperm DNA is limited, the Biomek® 2000/DNA IQ™ System generates DNA of similar, and sometimes better, quality and yield than that obtained by manual extraction of a duplicate sample. Because of the adaptability of the Biomek® 2000/ DNA IQ™ System, this instrument has the potential to handle future applications of emerging cell separation technologies. It may be possible on a robotic platform, to separate sperm cells from non-sperm cells with the use of an anti-sperm antibody conjugated to a magnetic bead. One can envision a completely automated system where both cell separation and DNA extraction are performed on the same robot. The Biomek® 2000/DNA IQ™ System may be uniquely poised to proceed with that application when the technology becomes available.

The time saved when compared with manual extraction, as well as the ability to extract a variety of tissue and cell types, makes the Biomek® 2000/DNA IQ™ System attractive for casework applications. Further validation work on the Biomek® 2000/DNA IQ™ System must be performed in order to complete our evaluation and validation prior to its application for forensic casework. Although no contamination of the samples was detected after modifying the method and changing the format, special circumstances might require the use of manual extraction methods, for example, when an evidentiary sample may be completely consumed due to limited available material.

ACKNOWLEDGMENTS

We would like to acknowledge the participation of other members of the Virginia Division of Forensic Science: Beth Ballard, Missy Baisden, Brian Covington, Shelley Smith and Colleen Young. We would also like to thank Allan Tereba and Dan Kephart at Promega Corporation for all their hard work at making the technology and this study possible.

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[a,b,c]Refer to the patent and disclaimer statements on page 2.

Exhibit B

33608 Ortega Highway
San Juan Capistrano, California 92690
949.728.4000

March 4, 2002

David Phelps
Promega
Genetic Identity
2800 Woods Hollow Drive
Madison, WI 53711

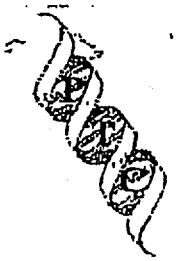
Dear Mr. Phelps,

We have evaluated DNA IQ system for isolation of DNA from clinical specimens (whole blood and buccal swabs). The IQ system showed satisfactory performance on both types of samples. It is highly tolerant to the variability of sample input quantity. It also demonstrated high consistency in the quantity of product DNA. This feature is extremely desirable to us as buccal swab samples can have significant variations in starting material affected by collection procedure and skills. In addition, DNA samples isolated using the DNA IQ system have shown satisfactory performance in the downstream applications and have excellent stability as well.

If there is any question in regards to our experience with the DNA IQ system, please do not hesitate to contact me.

Sincerely,

Weimin Sun, Ph.D., ABMG.
Scientific Director
Molecular Genetics Department
Quest Diagnostics Nichols Institute
33608 Ortega Highway
San Juan Capistrano, CA 92690
(949) 728-4498 (voice)
(949) 728-4874 (fax)



Paternity Testing Corporation

Fast, Confidential DNA *Analysis*

February 25, 2002

David Phelps
Promega, Genetic Identity
2800 Woods Hollow Drive
Madison, WI 53711

VIA FACSIMILE: (608) 273-6455

Dear Mr. Phelps:

Lisa Lane requested that I send you a brief letter about our experience using the DNA IQ extraction method.

PTC has been extremely interested in automating DNA extractions from buccal swabs. Because of the nature of buccal swabs it has historically been extremely difficult if not impossible to work with them in a 96 well automated format. Swabs absorbed liquid and had to be spun through a basket in order to recover the lysis buffer. Swabs had to be removed by hand one at a time. There were many transfers in and out of centrifuges. It was a very labor intensive process to carry out the extractions. It was possible to automate the extractions, but that required large volumes and could not be carried out in a 96 well format.

Our goal was to be able to put a buccal swab into a 96 well tray and never have to touch it again. DNA IQ allows for extractions to be carried out in the 96 well format from the time the specimens are placed into a 96 well deep well tray until the DNA is transferred to the amplification tray.

DNA IQ also has the advantage of eliminating the need to quantify the DNA. Buccal swabs vary greatly in DNA content. Since the DNA multiplexes are somewhat concentration sensitive, it was necessary to get a rough quantification of the amount of DNA present then diluting to appropriate volumes. There is no need to quantify the DNA using the DNA IQ extraction method. The concentration of DNA is consistent from well to well. By using 1µl of the isolated DNA we are able to get consistent results from virtually all specimens.

3501 Berrywood Dr. • Columbia, MO 65201
Toll Free 1-888-837-8323 • Phone: (573) 442-9948 • Fax: (573) 442-9870
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DNA IQ is also much faster than most extraction methods. It takes approximately 3 hours from the time the swabs are placed into the tray until the DNA is pipetted into the amplification tray. There is less than 30 minutes of analysts time involved in the entire process. Using our traditional extraction method it takes 6 or 7 hours to prepare 96 samples for amplification and more than 4 hours of actual hands on time by the analysts.

Our lab has nearly completed the final stages of testing and validation of DNA IQ. We plan to be online using DNA IQ in March.

Yours truly,

Kim Gorman

Kim Gorman
President

Exhibit D

COMMONWEALTH of VIRGINIA

DEPARTMENT OF CRIMINAL JUSTICE SERVICES

DIVISION OF FORENSIC SCIENCE
CENTRAL LABORATORY
A Nationally Accredited Laboratory

P.O. BOX 999
RICHMOND, VIRGINIA 23218
(804) 786-4707

February 25, 2002

David Phelps
Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399

Dear Mr. Phelps,

In order to aid the DNA examiner in the extraction of casework samples, the Virginia Division of Forensic Science, Forensic Biology Section, for the past 6 months has worked with scientists and engineers from the Promega Corporation and Beckman Coulter to evaluate, modify and validate the Beckman Coulter Biomek® 2000 Workstation in conjunction with the Promega DNA IQ™ Isolation System. Currently each DNA examiner spends between 6 and 8 hours purifying the DNA from an average sexual assault case consisting of 3 to 5 evidence samples. Because of the adaptability of the Promega DNA IQ™ Isolation System the DNA purification can be automated permitting the user to walk away once the samples are setup in the robot.

In the time it would takes 4 DNA case examiners (i.e. between 24 and 32 person hours) to manually purify the DNA from evidence samples from 3 to 5 cases, using an organic extraction procedure, the robot can accomplish in under 2 hours due to the speed of the Promega DNA IQ™ purification process paired with the Beckman Coulter Biomek® 2000 Workstation. Therefore, with as little as 5 to10 minutes of a technician's time to setup the instrument, the Virginia Division of Forensic Science, Forensic Biology Section can improve the turnaround time of cases with minimum impact on the casework examiner.

Utilizing the chemistry of the Promega DNA IQ™ Isolation System in combination with the Beckman Coulter Biomek® 2000 Workstation or similar instrumentation can greatly increase a laboratory's throughput capabilities for analyzing forensic casework samples. Thus in turn

Exhibit E

permitting the Forensic DNA community to provide a better service to the law enforcement agencies throughout the United States to help solve crimes against persons and property.

Yours sincerely,

Jeffrey D. Ban
Forensic Biology
Section Chief

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The R&D 100 Awards recognize the most promising new products, processes, materials, or software developed throughout the world and introduced to the market the previous year. Awards are based on each achievement's technical significance, uniqueness, and usefulness compared to competing projects and technologies.

DOE R&D 100 Awards
2009

Following are news releases and information about R&D 100 Awards won by the Department of Energy and its national laboratories in 2009:

07/24/09	DOE-Funded Research Projects Win 46 R&D 100 Awards for 2009
07/23/09	NETL Technologies Earn Prestigious R&D 100 Awards
07/23/09	Ames Laboratory Scientists Win R&D 100 Award for Virtual Engineering Software
07/23/09	Brookhaven Lab and Hybridyne Imaging Technologies, Inc., Win R&D 100 Award for Developing a Compact Gamma Camera for Imaging of Prostate Cancer
07/22/09	NREL Research Garners Three Prestigious R&D 100 Awards
07/21/09	INL wins three R&D 100 Awards
07/20/09	Sandia wins five R&D 100 awards
07/20/09	PNNL wins 2 R&D 100 awards for human health, renewable energy advances
07/20/09	ORNL researchers win eight R&D 100 awards
07/20/09	LLNL scientists and engineers garner eight awards for top industrial innovations
07/20/09	Berkeley Lab Wins Four 2009 R&D 100 Awards
07/20/09	Argonne researchers win four R&D 100 awards

DOE R&D 100 Awards
1992-2008

The following list details the number of R&D 100 Awards won by

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R&D 100 Awards

For 42 years, the prestigious R&D 100 Awards, sponsored by R&D Magazine, have been helping companies provide the important initial push a new product needs to compete successfully in the marketplace.

The winning of an R&D 100 Award provides a mark of excellence known to industry, government, and academia as proof that the product is one of the 100 most technologically significant new products of the year.

High Temperature Superconductivity

- High-Performanc e LMO-enabled, High Temperature Superconducting Wires, 2007 - Oak Ridge National Laboratory, SuperPower
- Flexible Superconducting Tape, 2003 - Los Alamos National Laboratory
- RABiTS Superconducting Wire Technology, 1999 - Oak Ridge National Laboratory
- High Temperature Superconductor Wire Technology, 1996 - Advanced Superconductor Corporation

ATTACHMENT 3

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 204 741 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
20.10.2004 Bulletin 2004/43

(21) Application number: **00955744.8**

(22) Date of filing: **18.08.2000**

(51) Int Cl.[7]: **C12N 15/10**, C12Q 1/68

(86) International application number:
PCT/US2000/022827

(87) International publication number:
WO 2001/014590 (01.03.2001 Gazette 2001/09)

(54) **SIMULTANEOUS ISOLATION AND QUANTITATION OF DNA**

SIMULTANE ISOLIERUNG UND QUANTIFIZIERUNG VON DNA

ISOLEMENT ET QUANTIFICATION SIMULTANES D'ADN

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **20.08.1999 US 377986**

(43) Date of publication of application:
15.05.2002 Bulletin 2002/20

(60) Divisional application:
04018521.7

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(56) References cited:
WO-A-98/31840

EP 1 204 741 B1

ATTACHMENT 4

Printed by Jouve, 75001 PARIS (FR)

Table 13 (continued)

Samples 10µl	Raw #	Corr #	Conc	Yield (ng)
#7	117	80	1.76	352
#8	100	63	1.39	277.2
#9	126	89	1.96	391.6

Using the PicoGreen Quantitation data, the average yield was 418 ng. The lowest point was 250.8 ng or 60% of the average. The highest point was 959 ng (#6 - probably outlier point) or 229% of the mean. If this point is disregarded in the calculation of the mean, the mean is 350 ng, the lowest point is 250 ng or 71 % of the mean and the highest point is 510 ng or 145% of the mean.

Claims

1. A method for isolating a defined quantity of a DNA target material from other cell components in a medium by:

   (a) providing a medium including the DNA target material and other cell components;
   (b) providing a discrete quantity of a silica-containing solid support capable of reversibly binding a definable quantity of DNA target material, the amount of DNA target material provided in step (a) being in excess of the binding capacity of the silica-containing solid support;
   (c) forming a complex of the silica-containing solid support and the DNA target material by combining the silica-containing solid support and the medium;
   (d) removing the complex with the DNA target material from the medium; and
   (e) separating the DNA target material of step (c) from the complex, whereby a defined quantity of the DNA target material is obtained.

2. A method according to claim 1, wherein the silica-containing solid support is silica magnetic particles, and the step of removing the complex with the DNA target material from the medium is accomplished by application of an external magnetic field.

3. The method of claim 2, wherein the silica magnetic particles are siliceous-oxide coated magnetic particles.

4. The method of either claim 2 or claim 3 wherein the silica magnetic particles are porous.

5. The method of either claim 2 or claim 3, wherein the silica magnetic particles are non-porous.

6. The method of any one of claims 2 to 5, wherein the medium includes a chaotropic salt.

7. The method of claim 6, wherein the chaotropic salt comprise guanidine thiocyanate.

8. The method of either claim 6 or claim 7, wherein the concentration of chaotropic salt in the mixture formed in step (c) is between 0.1 and 7 M.

9. The method of any one of claims 2 to 8, wherein the DNA target material provided in step (a) is the product of a polymerase chain reaction.

10. The method of any one of claims 2 to 9, wherein the DNA target material is genomic DNA.

11. The method of any one of claims 2 to 9, wherein the DNA target material is plasmid DNA.

12. The method of claim 10 further comprising analyzing the eluted genomic DNA in a DNA typing process.

13. The method of any one of claims 2 to 12, wherein the medium is a solid support containing the DNA target material and wherein the DNA target material is isolated from the solid support prior to step (c) by combining the solid support with a mixture comprising a chaotropic salt.

14. The method of claim 13 wherein the solid support is a paper.

15. The method of either claim 13 or claim 14, wherein the mixture is heated to a temperature of from about 60° to about 100°C.

16. The method of any one of claims 2 to 15, further comprising sequencing at least a portion of the eluted DNA target material.

17. The method of any one of claims 2 to 16, further comprising a step of washing the complex after removal from the medium, before eluting the DNA target material from the complex.

18. The method of claim 17, wherein the complex is washed using a wash solution comprising an alcohol and a salt.

19. The method of any one of claims 2 to 18, wherein the DNA target material eluted in step (e) is eluted with water.

Patentansprüche

1. Verfahren zur Isolation einer definierten Menge DNA-Zielmaterial von anderen Zellkomponenten in einem Medium durch

   (a) Bereitstellung eines Mediums, welches das DNA-Zielmaterial und andere Zellkomponenten umfasst;
   (b) Bereitstellung einer diskreten Menge eines silicahältigen festen Trägers, der zur reversiblen Bindung einer definierbaren Menge DNA-Zielmaterial fähig ist, wobei die Menge an DNA-Zielmaterial, die in Schritt (a) bereitgestellt wird, die Bindungskapazität des silicahältigen festen Trägers übersteigt;
   (c) Bildung eines Komplexes aus dem silicahältigen festen Träger und dem DNA-Zielmaterial durch Kombination des silicahältigen festen Trägers und des Mediums;
   (d) Entfernung des Komplexes mit dem DNA-Zielmaterial aus dem Medium; und
   (e) Trennung des DNA-Zielmaterials von Schritt (c) vom Komplex, wodurch eine definierte Menge des DNA-Zielmaterials erhalten wird.

2. Verfahren nach Anspruch 1, worin der silicahältige feste Träger aus magnetischen Silicateilchen besteht und der Schritt des Entfernens des Komplexes mit dem DNA-Zielmaterial aus dem Medium durch Anlegen eines externen Magnetfeldes durchgeführt wird.

3. Verfahren nach Anspruch 2, worin die magnetischen Silicateilchen mit silikatischem Oxid beschichtete Magnetteilchen sind.

4. Verfahren nach Anspruch 2 oder Anspruch 3, worin die magnetischen Silicateilchen porös sind.

5. Verfahren nach Anspruch 2 oder Anspruch 3, worin die magnetischen Silicateilchen nicht porös sind.

6. Verfahren nach einem der Ansprüche 2 bis 5, worin das Medium ein chaotropes Salz umfasst.

7. Verfahren nach Anspruch 6, worin das chaotrope Salz Guanidinthiocyanat umfasst.

8. Verfahren nach Anspruch 6 oder Anspruch 7, worin die Konzentration des chaotropen Salzes in dem in Schritt (c) hergestellten Gemisch zwischen 0,1 und 7 M liegt.

9. Verfahren nach einem der Ansprüche 2 bis 8, worin das in Schritt (a) bereitgestellte DNA-Zielmaterial das Produkt einer Polymerasekettenreaktion ist.

10. Verfahren nach einem der Ansprüche 2 bis 9, worin das DNA-Zielmaterial genomische DNA ist.

11. Verfahren nach einem der Ansprüche 2 bis 9, worin das DNA-Zielmaterial Plasmid-DNA ist.

12. Verfahren nach Anspruch 10, das außerdem eine Analyse der eluierten genomischen DNA in einem DNA-Typisierungsverfahren umfasst.

ATTACHMENT 4

K. NAKAMATSU
(1895-1973)

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November 20, 2009

TJW

Michael Best & Friedrich LLP
IP Docketing Department
Attention: Jill A. Fahrlander, Esq.
P.O. Box 1806
Madison, WI 53701-1806
U.S.A.

VIA ~~FACSIMILE~~ EMAIL ONLY
IMMEDIATE REPLY REQUESTED

Re: Japanese Patent Application No. 2001 - 518457
SIMULTANEOUS ISOLATION AND QUANTITATION OF DNA
PROMEGA CORPORATION
Your Ref : 016026-9043-JP00
Our File : X1J - 0158

Dear Sirs:

An official decision of grant for patent has been issued in connection with the above application and the issue fee covering the first to the third annuities is to be paid by December 19, 2009.

The cost of paying the issue fee is ¥ 29,100 as the annuities plus ¥ 32,000 as the attorney's service fee.

Please give us your instructions on whether to pay well before the above date. Unless otherwise instructed to the contrary by the above date, we will pay the issue fee so that the application may proceed to patent to avoid an inadvertent loss of rights in the application.

~~A certified copy of the decision of grant is being sent to you with the original of this letter.~~

Very truly yours,

N. Ogawa

DOCKETED
Date: 11-20-2009 jdk
Also on JAF, TRB's docket

ATTACHMENT 5

整理番号:　　発送番号:767821　発送日:平成21年11月19日　　1

特許査定 Decision of Grant

特許出願の番号	特願２００１－５１８４５７
起案日	平成２１年１１月１３日
特許庁審査官	滝口　尚良　　８９２７　４Ｎ００
発明の名称	ＤＮＡの同時単離及び定量化
請求項の数	３７
特許出願人	プロメガ　コーポレイション
代理人	中村　稔（外　６名）

[前置審査]
原査定を取消す。
この出願については、拒絶の理由を発見しないから、特許査定をします。

上記はファイルに記録されている事項と相違ないことを認証する。
認証日　平成21年11月16日　経済産業事務官　吉越　誠

注意：この書面を受け取った日から３０日以内に特許料の納付が必要です。

ATTACHMENT 5

1．出願種別　　通常
2．参考文献　　有
3．特許法第３０条適用　　無
4．発明の名称の変更　　無

5．国際特許分類（ＩＰＣ）

Ｃ１２Ｎ　１５／００	Ａ,
Ｇ０１Ｎ　３３／５０	Ｐ＝
Ｃ１２Ｑ　　１／６８	Ａ

6．菌寄託

7．出願日の遡及を認めない旨の表示

参考情報

特許出願の番号　　特願２００１－５１８４５７

1．調査した分野（ＩＰＣ，ＤＢ名）

Ｃ１２Ｎ　１５／０９
Ｇ０１Ｎ　３３／５０
Ｃ１２Ｑ　　１／６８
ＣＡ／ＭＥＤＬＩＮＥ／ＥＭＢＡＳＥ／ＢＩＯＳＩＳ（ＳＴＮ）

2．参考特許文献

国際公開第９８／０３１８４０　　（ＷＯ，Ａ１）

3．参考図書雑誌

Japanese Patent Application No. 2001-518457
SIMULTANEOUS ISOLATION AND QUANTITATION OF DNA
ALLOWED CLAIMS

1. A method for isolating a defined quantity of a DNA target material from other material in a medium by:

a. providing a medium including the DNA target material;

b. providing a discrete quantity of a silica-containing solid support capable of reversibly binding a definable quantity of the DNA target material, the amount of DNA target material provided in step (a) being in excess of the binding capacity of the solica-containing solid support;

c. forming a complex of the silica-containing solid support and the DNA target material by combining the silica-containing solid support and the medium;

d. removing the complex with the DNA target material from the medium; and

e. separating the DNA target material of step (c) from the complex, whereby a defined quantity of the DNA target material is obtained.

2. A method for isolating a defined quantity of a DNA target material from other material in a medium by:

a. providing a medium including the DNA target material;

b. providing a discrete quantity of silica magnetic particles capable of reversibly binding a definable quantity of the DNA target material, the amount of DNA target material provided in step (a) being in excess of the binding capacity of the particles;

c. forming a complex of the silica magnetic particles and the DNA target material by combining the silica magnetic particles and the medium;

d. removing the complex with the DNA target material from the medium by application of an external magnetic field; and

e. separating the DNA target material of step (c) from the complex by eluting the DNA target material, whereby a defined quantity of the DNA target material is obtained.

3. The method of claim 2, wherein the silica magnetic particles are porous.

ATTACHMENT 5

4. The method of claim 2, wherein the silica magnetic particles are nonporous.

5. The method of claim 2, wherein the silica magnetic particles are siliceous-oxide coated magnetic particles.

6. The method of claim 2, wherein the medium includes a chaotropic salt.

7. The method of claim 6, wherein the chaotropic salt comprises guanidine thiocyanate.

8. The method of claim 2, wherein the DNA target material provided in step (a) is the product of a polymerase chain reaction.

9. The method of claim 2, wherein the DNA target material is genomic DNA.

10. The method of claim 2, wherein the DNA target material is plasmid DNA.

11. The method of claim 9, further comprising analyzing the eluted genomic DNA in a DNA typing process.

12. The method of claim 2, wherein the medium is a solid support containing the DNA target material and wherein the DNA target material is isolated from the solid support prior to step (c) by combining the solid support with a mixture comprising a chaotropic salt.

13. The method of claim 12, wherein the solid support is a paper.

14. The method of claim 12, wherein the mixture is heated to a temperature of from about 60°C to about 100°C.

15. The method of claim 2, further comprising sequencing at least a portion of the eluted DNA target material.

16. The method of claim 2, further comprising a step of washing the complex after removal from the medium, before eluting the DNA target material from the complex.

17. The method of claim 16, wherein the complex is washed using a wash solution comprising an alcohol and a salt.

18. The method of claim 2, wherein the DNA target material eluted in step (e) is eluted with water.

19. A method of isolating a defined quantity of a DNA target material from other materials in a medium comprising the steps of:

a. providing a medium containing the DNA target material;

b. providing a discrete quantity of silica magnetic particles with the capacity to reversibly bind a definable quantity of the DNA target material per milligram of particle, the amount of DNA target material provided in step (a) being in excess of the binding capacity of the particles;

c. forming a mixture comprising the medium, the silica magnetic particles, and a chaotropic salt, wherein the chaotropic salt concentration in the mixture is sufficient to cause the DNA target material to adhere to the particles;

d. incubating the mixture until at least some of the DNA target material is adhered to the silica magnetic particles;

e. removing the silica magnetic particles and the adhered DNA target material from the mixture using an external magnetic force; and

f. eluting the DNA target material of step (e) from the silica magnetic particles by exposing the particles to an elution solution, whereby a defined quantity of the DNA target material is obtained.

20. The method of claim 19, wherein the DNA target material is genomic DNA.

21. The method of claim 19, wherein the DNA target material is plasmid DNA.

22. The method of claim 19, further comprising sequencing at least a portion of the eluted DNA target material.

23. The method of claim 19, wherein the chaotropic salt comprises guanidine thiocyanate.

24. The method of 19, wherein the concentration of chaotropic salt in the mixture formed in step (c) is between about 0.1 M and 7 M.

25. The method of claim 19, wherein the silica magnetic particles are porous.

26. The method of claim 19, wherein the silica magnetic particles are nonporous.

27. The method of claim 19, further comprising a step of washing the silica magnetic particles after removal from the medium, before eluting the DNA target material from the particles.

28. The method of claim 19, wherein the particles are washed using a wash solution comprising an alcohol and a salt.

29. The method of claim 19, wherein the elution solution is water.

30. A method of determining a calibration model for quantitating a DNA target material in a sample type of interest, the method comprising:

a. providing a first medium, wherein the first medium includes a first quantity of the sample type of interest;

b. providing a second medium, wherein the second medium includes a second quantity of the sample type of interest, wherein the second quantity is greater than the first quantity of the sample type of interest;

c. mixing a first discrete quantity of silica magnetic particles with the first medium, wherein the silica magnetic particles are capable of reversibly binding a first quantity of the DNA target material, thereby forming a first complex of the silica magnetic particles and the DNA target material from the first medium, the discrete quantity of the sample type of interest in the first medium containing DNA target material in excess of the binding capacity of the particles mixed with the first medium;

d. mixing a second discrete quantity of silica magnetic particles with the second medium, wherein the silica magnetic particles are capable of reversibly binding a second quantity of the DNA target material, thereby forming a second complex of the silica magnetic particles and the DNA target material from the second medium, the discrete quantity of the sample type of interest in the second medium containing DNA

target material in excess of the binding capacity of the particles mixed with the second medium;

e. removing the first complex from the first medium and the second complex from the second medium by application of an external magnetic field;

f. separately eluting the DNA target material from the first complex and second complex, producing a first eluent of isolated DNA target material from the first complex and a second eluent of isolated DNA target material from the second complex; and

g. determining the amount of DNA target material in the first eluent and in the second eluent.

31. The method of claim 30, wherein the discrete quantity of particles provided in step (c) is the same quantity as the discrete quantity of particles provided in step (d).

32. A method of claim 1, wherein the medium is a solid medium.

33. The method of claim 1, wherein the medium is a paper.

34. The method of claim 1, wherein the medium is a swab.

35. The method of claim 1, wherein the medium is a forensic sample.

36. The method of claim 1, wherein step (c) is performed in the presence of a chaotropic salt.

37. The method of claim 1, wherein the medium is contacted with a chaotropic salt prior to step (c) and the contacted medium is heated to a temperature of from about 60°C to about 100°C.

Q:\CLIENT\016026\9043\B2099502.1

Products

Overview

Product Listing A - Z

Applications Listing A - Z

Product Catalog

General Research

Clinical Laboratory

Genetic Analysis

Drug Discovery Research

Immunology

Lab Automation

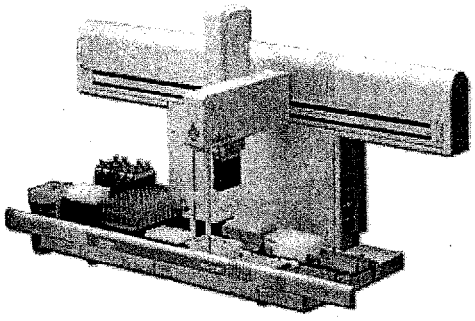
Disease Management and Primary Care

Biomek® 2000 Laboratory Automation Workstation

Printer Friendly

Related Product Information
Select

Years of reliability, versatility and innovation have made the Biomek 2000 Laboratory Automation Workstation the benchmark system for BioRobotics in Pharmaceutical, Human Genome and Bioresearch laboratories worldwide. Virtually any liquid-handling procedure can be automated with the Biomek 2000, controlled by powerful and easy-to-use BioWorksâ„¢ software.



Biomek 2000 Laboratory Automation Workstation

Product Features

The Biomek 2000 Laboratory Automation Workstation meets the demands of rapidly changing life science technology with simple, intelligent automation of liquid-handling tasks. Pipetting, diluting and dispensing operations are performed quickly, easily and automatically. The modular platform allows expansion of system capability to include plate heating and cooling, plate washing, high-density transfers, photometric measurement and high-capacity operation. The entire system is controlled by powerful and user-friendly BioWorks software with an intuitive graphical interface.

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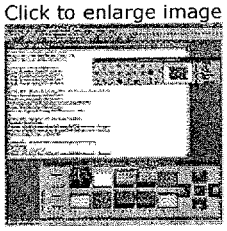

BioWorks Software

Intelligent Automation Provides Fast, Simple Setup

- Click Here for more information on Biomek 2000 Software
- BioWorks software quickly guides the operator step-by-step through simple graphic menus and prompts
- Workstation methods are easily configured to follow laboratory techniques
- All methods are validated for consistency
- System configurations are automatically verified
- Potential conflicts are detected by the software

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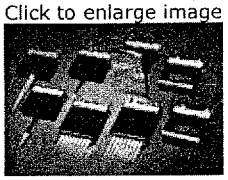

Biomek Tools

Interchangeable Liquid-Handling Tools Allow Easy Modification to Meet Expanding Research Requirements

- Pipette tools transfer from 1 Âµ to 1 mL with high precision
- Faster throughput achieved with multichannel tools
- Inefficient wash steps of probe-based systems are avoided by using disposable pipette tips with proven contamination-free liquid transfers
- Effective tracking of liquid levels is achieved with patented sonic liquid-sensing technology which accurately detects microliter volumes of both conductive and non-ionic liquids

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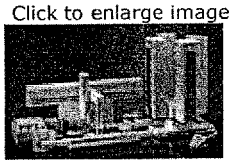

The Biomek Stacker Carousel provides external supply and storage of microplates, deep-well plates, and P20 and P250 pipette tips for the Biomek 2000 Laboratory Automation Worstation. This increases capacity and walk-away operation of the Biomek 2000 for all applications.

ATTACHMENT 6